Old Wine in New Bottles: Reviving Old Therapies for Alopecia Areata Using Rodent Models

Liren Tang, John P. Sundberg*, Harvey Lui and Jerry Shapiro
Division of Dermatology, University of British Columbia, Vancouver, Canada; *The Jackson Laboratory, Bar Harbor, Maine, USA

Alopecia areata is regarded as a tissue-restricted autoimmune disease of hair follicles in which follicular activity is arrested because of the continued activity of lymphocytic infiltrates. Actual loss of hair follicles does not occur, even in hairless lesions. A variety of immunomodulating therapies, including contact sensitizers and immunomodulators, are part of the usual armamentarium for this disorder. None of these treatments have been consistent in their efficacy, and many have untoward side effects. Nevertheless, their uses in valid animal models provide a tool to dissect out molecular mechanisms of therapeutic effects. For several decades, both mechlorethamine (for the treatment of cutaneous T cell lymphoma) and anthralin (for the treatment of psoriasis) have been used successfully. When these therapies were tested in rat and mouse alopecia areata models, we found anthralin and mechlorethamine to be the most effective topical modalities, respectively. The underlying cellular mechanisms may act through targeting infiltrative lymphocytes, and the molecular mechanisms may involve specific cytokine expression changes. These visible, accessible, and unilaterally treated animal model systems are ideal for studying novel alopecia areata therapies, particularly in terms of their *in vivo* molecular mechanisms of action. ***Keywords: anthralin/autoimmunity/cytokines/mechlorethamine. JID Symposium Proceedings 8:212–216, 2003***

Pathogenesis of alopecia areata Alopecia areata (AA) is a common inflammatory disease of the hair follicle, affecting 1.7% of the population, that can clinically present as patchy to complete hair loss (Safavi *et al*, 1995); it is widely regarded as an autoimmune disease (McDonagh and Messenger, 1996; McElwee *et al*, 1999a; Randall, 2001). The pathogenic role of infiltrative T lymphocytes has been directly demonstrated by cell transfer experiments (McElwee *et al*, 1996, 1999c; Gilhar *et al*, 1998, 2001, 2002). These infiltrating T cells preferentially attack anagen hair bulbs in the active lesions of AA (Perret *et al.*, 1984; Todes-Taylor *et al*, 1984). Loss of hair follicles does not actually occur even in hairless lesions. Rather, in active AA the follicles no longer produce visible hair fiber as a result of the continued activity of the lymphocytic infiltrate. As with all other autoimmune disorders, the cause of AA is multifactorial. Analysis of family history suggests a genetic component for approximately 20% of cases, but to date there is no concrete evidence for exactly which genes are responsible. A number of cytokines, growth factors, and regulatory molecules have been postulated as having significant roles in AA, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interferon-γ (IFN-γ), IL-1 receptor (IL-1R), calcitonin gene–related protein (CGRP), and other neuropeptides (Hoffman *et al*, 1994; Cork *et al*, 1995, 1996; Hoffmann and Happle, 1995; Philpott *et al*, 1996; Carroll *et al*, 1997).

Alopecia areata therapies Treatments for alopecia areata include contact sensitizers, immunomodulators, and biological response modifiers (Hoffmann and Happle, 1996; Shapiro and Price, 1998; Shapiro, 2002). Unfortunately, however, none of these therapeutic modalities consistently produce successful results and many have untoward side effects. Topical anthralin treatment (Nelson and Spielvogel, 1985; Fiedler-Weiss and Buys, 1987) and topical nitrogen mustard (Arrazola *et al*, 1985; Bernardo *et al*, in press) are effective on AA patients, but results have been inconsistent. Exactly how these agents result in hair regrowth and why only certain patients respond has not been elucidated. Their successful application in a valid animal model would provide a means to test the efficacy and safety of therapeutic reagents as well as dissect out their mechanism of action in order to develop more targeted therapy with a more favorable side-effect profile for AA patients.

Rodent models for alopecia areata Animal models provide the means whereby new forms of treatment can be developed and tested. There are two rodent models for AA. Aging C3H/HeJ mice develop a nonscarring alopecia with clinical and pathologic features similar to those of human AA (Sundberg *et al*, 1994, 1996). The onset of hair loss is typically observed as early as four months of age in females and six to twelve months in males. Expression of hair loss rises to 20% in some colonies of mice (Sundberg *et al*, 1994). The hair loss phenotype can be reproduced by whole-skin grafting of the AA-affected mouse skin onto normal C3H/HeJ mice (McElwee *et al*, 1999b). The other rodent model, the Dundee experimental bald rat (DEBR), is a hooded rat strain that also exhibits hair loss lesions similar to

Manuscript received December 30, 2002; revised February 12, 2003; accepted for publication March 11, 2003
Correspondence to: Jerry Shapiro, Division of Dermatology, University of British Columbia, Vancouver General Hospital, 835 West 10th Avenue, Vancouver, BC, Canada V5Z4E8. Email: shapiro@interchange.ubc.ca
Abbreviations: AA, alopecia areata; DEBR, Dundee experimental bald rat; IFN, interferon; IL, interleukin; RPA, RNase protection assay; RT-PCR, reverse transcription polymerase chain reaction; TNF, tumor necrosis factor.

those observed in human AA. All DEBR rats develop a normal coat of hair from birth up to four months, but up to 30% of males and 80% of females exhibit hair loss on their heads, which then typically progresses to alopecia of the flanks and the entire body (Michie *et al*, 1991; Zhang and Oliver, 1994). We and other investigators have shown that various therapies can stimulate hair regrowth in AA-affected rats and mice (Oliver and Lowe, 1995; McElwee *et al*, 1997; Freyschmidt-Paul *et al*, 1999, 2001; Shapiro *et al*, 1999; Tang *et al*, 2003a, b).

ANTHRALIN IN THE TREATMENT OF AA RODENTS

Anthralin is one of the oldest, most effective, and widely used therapeutic agents for the treatment of psoriasis (Wiegrebe and Muller, 1995; Harris, 1998). It has now been shown to be successful for some patients with patchy AA (Fiedler-Weiss and Buys, 1987). Anthralin's therapeutic effects on AA were tested on both rodent AA models.

Anthralin therapy in AA-affected C3H/HeJ mice Affected C3H/HeJ mice were treated unilaterally daily for five days per week on the dorsal skin with 0.2% anthralin ointment; the contralateral side was treated with the vehicle alone. Significant hair regrowth was observed on the treated side in 64% of the mice (Tang *et al*, 2003a). Four mice displayed near complete replacement of hairs of normal density and length. Hair regrowth was observed unilaterally on the anthralin-treated side as early as one week after the initiation of treatment in one mouse. By week 10, there was extensive hair replacement with normal hair density and quality on the anthralin-treated side in the responding mice. The vehicle-treated side either remained unchanged or continued to demonstrate hair loss compared to baseline. The nonresponders showed either no hair regrowth as compared to the control side or hair loss on both sides. In anthralin-treated skin, local dermatitis with erythema and hyperpigmentation was observed as early as two to three days after starting treatment and continued for the duration of the study.

Anthralin therapy in DEBR rats AA-affected rats were treated similarly on the dorsal surface, except with 0.1% anthralin ointment. After six to eight weeks of treatment, follicular activity was reversed with clear regrowth over nearly all of the treated side in all rats whereas the control side remained bald. Visible hair regrowth was observed as early as one week after the onset of treatment in some rats.[1] Among the drugs tested on this AA model in our lab, topical anthralin appears to be the most promising and exhibits near perfect hair regrowth in all of the DEBR rats tested (**Fig 1**). The effect of anthralin on normal Wistar rats after shaving was also tested. No indication of its hair stimulatory effects on normal rats was observed after a similar course of treatment, indicating that anthralin's therapeutic effects on hair regrowth are specific to AA-affected rats.

Immunohistochemical studies in rats Immunohistochemical analysis revealed that infiltrating lymphocytes in AA-affected DEBR rat skin were mainly composed of CD8+ cells along with very few CD4+ cells. On the vehicle-treated side, CD8+ cells were mainly present around the follicular periphery and also penetrated the intrafollicular peribulbar area. In contrast, under anthralin treatment the CD8+ cells were mainly interfollicular and distributed more uniformly in the dermis. It appeared that the distribution of these cells changed between the two sides but that the absolute numbers did not (Tang *et al*, submitted). Interestingly, the pattern of ICAM-1 (CD54 antibody) expression was very similar to that of CD8 and also showed consistent redistribution in all rats tested (unpublished data). The number of CD4+ cells in the tested rats was low, and there was no appreciable difference between the two sides in either the number or the location of the CD4+ cells.

[1]Tang L, Cao L, Lui H, Shapiro J: Restoration of follicular activity by anthralin in DEBR rats affected by alopecia areata. *Exp Dermatol* (submitted).

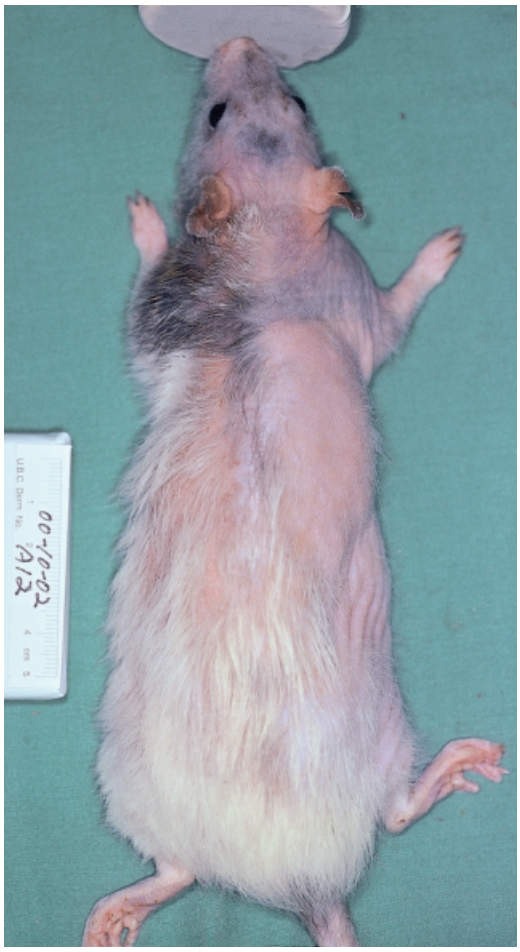

Figure 1. A female DEBR rat was successfully treated unilaterally with anthralin. The rat was treated on the left side with anthralin ointment and on the right side with the vehicle for eight weeks. The treated side showed near normal hair regrowth, whereas the left side remained alopecic.

Cytokines modulated by anthralin in AA-affected rodents In skin, a large number of cytokines are produced under both normal and pathologic conditions, and they play important roles in the pathogenesis of inflammatory skin diseases. The mechanism of anthralin's therapeutic effects in AA is unclear. Various cytokines have been associated with the success of anthralin in the treatment of psoriasis (Mrowietz *et al*, 1997; Chodorowska, 1998; Lange *et al*, 1998), and certain pro-inflammatory cytokines might be involved in the pathogenesis of AA (Cork *et al*, 1995; Hoffmann and Happle, 1995; Cork *et al*, 1996; Philpott *et al*, 1996; Carroll *et al*, 1997). To explore the possible molecular mechanisms of anthralin's therapeutic effects on hair restoration in AA-affected C3H/HeJ mice and DEBR rats, we performed RNase protection assays (RPA) and real-time reverse transcription polymerase chain reactions (RT-PCR) on the biopsies from anthralin-treated and vehicle-treated control sides. In mice, RPA showed that the majority of the responding mice displayed decreased expression of TNF-α and TNF-β on the treated sides. In contrast, nonresponders remained unchanged in the expression of TNF-α/β. Two abundantly expressed cytokines (IL-18 and IL-1Ra) and other weakly expressed cytokines (IL-1a,

Table I. Summary of cytokine expression mediated by anthralin and mechlorethamine in alopecia areata animal models. The levels of different cytokines were normalized to that of L32 from the same sample. The ratio of treated over control was used to determine the difference in the gene expression between two sides. The ratio of greater than 2 or less than 0.5 was considered as increased or decreased, respectively. The differences were statistically significant. Anything between was valued as no change

Cytokines	Animal: DEBR rat Therapy: Anthralin	Animal: C3H/HeJ AA mouse Therapy: Anthralin	Animal: C3H/HeJ AA Mouse Therapy: Meclorethamine
IFN-γ	decreased	not tested	decreased
TNF-α	decreased	decreased*	decreased
TNF-β	not tested	decreased*	decreased
IL-1α	increased	no change	no change
IL-1β	increased	no change	no change
IL-1Ra	increased	no change	no change
IL-10	increased	not detected	no change
IL-12	no change	no change	decreased
MIF-1	not tested	not tested	no change
IL-18	no change	no change	no change

*: only decreased in the responding mice, no change in the non-responders.

IL-1b, IL-10, and IL-12) in mouse skin did not show any significant differences between the two sides (**Table I**).

The pattern of cytokine expression in DEBR rat skin after anthralin treatment was different. IL-1a/b, IL-10, and IL-1Ra were increased by the treatment in all the rats tested. TNF-β mRNA was not consistently detected from the rat skin, and, interestingly, TNF-α and IFN-γ mRNA were reduced (**Table I**). Whether this differential cytokine profiling following anthralin treatment is associated with the different responses between the two animal models needs to be further investigated. Alternatively, each rodent species may represent a different type or subtype of AA with different mechanisms involved.

MECHLORETHAMINE THERAPY IN AA-AFFECTED C3H/HEJ MICE

Mechlorethamine was one of the first anticancer drugs developed, and it is still widely used in the treatment of lymphoma (Engert *et al*, 1999; Tesch *et al*, 2001). Its topical formulation is regularly used to treat cutaneous T cell lymphoma (Ramsay *et al*, 1995; Duvic *et al*, 1996; Esteve *et al*, 1999). The low-dose topical ointment has been shown to have some efficacy with AA patients (Arrazola *et al*, 1985; Bernardo *et al*, in press). We treated AA-affected mice unilaterally on the dorsal skin with mechlorethamine and, as a control, on the contralateral side with the vehicle ointment. After five to ten weeks of therapy, a full pelage of hair covered the entire mechlorethamine-treated side in all the mice whereas the vehicle-treated side showed either no change or continued hair loss (**Fig 2**). As a control, normal C3H/HeJ mice were shaved on the back and then treated the same way as AA-affected mice. No difference between mechlorethamine-treated and vehicle-treated sides in hair regrowth was observed in the normal mice after a similar course of treatment, indicating that mechlorethamine's therapeutic effects on hair regrowth are specific for AA-affected mice. For these mice, mechlorethamine appears to be the most effective treatment among the drugs tested to date (Freyschmidt-Paul *et al*, 1999, 2001; Shapiro *et al*, 1999; Tang *et al*, 2003b).

Immunohistochemistry revealed that both CD4+ and CD8+ lymphocytes were depleted by mechlorethamine treatment whereas, on the vehicle-treated control sides, CD4+ and CD8+ cells were easily observed in the peri- and intrafollicular areas. In an *in vitro* test on activated primary mouse T lymphocytes, we found that human T lymphocytes and cells from a lymphoma T cell line (Jurkat) were all much more susceptible to mechlorethamine's cytotoxic and proliferation inhibitory effects compared to all other native skin cells. These other skin cells included follicular and epidermal keratinocytes in addition to dermal and follicular dermal papillae fibroblasts (Tang *et al*, 2003b). The data strongly suggest that topical mechlorethamine therapy selectively targets the infiltrating lymphocytes *in vivo* in AA-affected skin to restore autoimmune-arrested hair follicle activity.

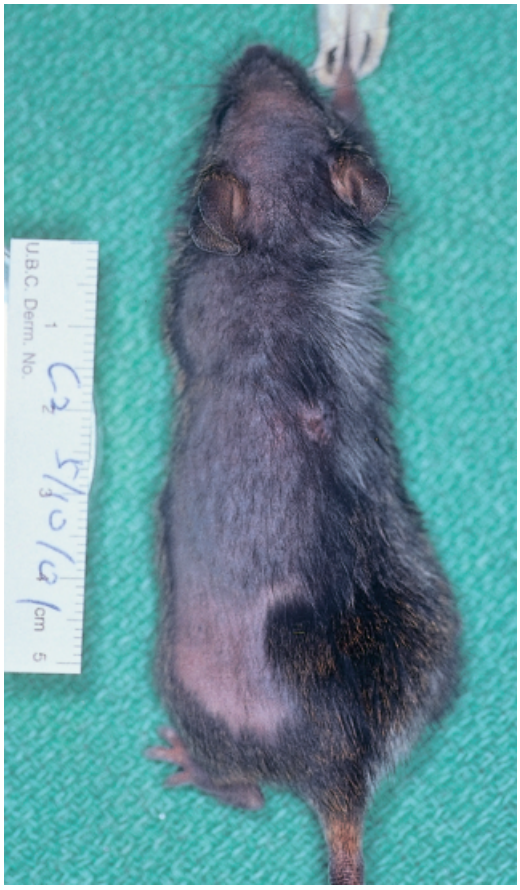

Figure 2. A female C3H/HeJ mouse was successfully treated unilaterally with mechlorethamine. The mouse was treated with mechlorethamine on the right side and with vehicle on the left side for eight weeks. The right side was covered with full pelage of hair; left side remained alopecic.

As mentioned before, the cytokines IL-1β, TNF-α, IFN-γ, and IL-1Ra have been implicated in the pathogenesis of AA (Hoffmann and Happle, 1995; Cork *et al*, 1996; Philpott *et al*, 1996; Carroll *et al*, 1997). By comparing the cytokine expression profiles between the mechlorethamine-treated and control sides, we discovered that TNF-α/β, IL-12, and IFN-γ were consistently inhibited by mechlorethamine (Tang *et al*, 2003b). Other cytokines, including IL-18, macrophage immigration factor-1 (MIF-1), and IL-1Ra, were abundantly expressed in mouse skin but did not exhibit any differences between the two sides. These results confirm that mechlorethamine-treated C3H/HeJ mice with an AA-like disease are a useful model in exploring the comparative molecular mechanisms of topical therapeutic agents for AA.

SUMMARY

No therapeutic modality consistently produces successful results in AA, and all have untoward side effects. How these reagents result in hair regrowth is not clear. Successful use in a valid animal model would provide a tool to dissect out their mechanisms of action in order to develop more targeted therapy with a milder side-effect profile for patients with AA. Our findings illustrate that AA-affected DEBR rats and C3H/HeJ mice are excellent models for studying therapy for AA and for exploring the corresponding molecular mechanisms of action. Among all reagents tested on these two rodent AA models, we found that anthralin and mechlorethamine are the most effective therapies.

At the cellular level, it appears that infiltrating lymphocytes are targeted by various immunomodulating reagents. The pathogenic roles of activated CD4+ and CD8+ lymphocytes have been demonstrated in both human and animal models (Becker *et al*, 1996; McElwee *et al*, 1996, 1999c; Gilhar *et al*, 1998, 2001, 2002; Bodemer *et al*, 2000). It is generally believed that CD8+ cells are more crucial than CD4+ cells. This hypothesis is supported by our data with anthralin and mechlorethamine in the rodent models. Anthralin might modulate the immune reaction around the hair follicles by redirecting the immune response away from the antigen responsible for AA, thus restoring arrested hair follicle activity. Mechlorethamine therapy might selectively target the infiltrating lymphocytes *in vivo* in AA-affected skin, as both CD4+ and CD8+ lymphocytes are eliminated. This is further supported by the differential sensitivity to mechlorethamine's cytotoxicity between lymphocytes and other skin and hair follicle cells *in vitro*.

Molecular mechanisms responsible for hair follicle restoration in AA are poorly understood. Altered expression of various cytokines has been shown to be associated with the pathogenesis of various autoimmune conditions, and some of them have served as therapeutic target molecules (Cork *et al*, 1995; Joosten *et al*, 1996; Cope, 1998; Firestein, 1998; Feldmann and Maini, 2001; Kassiotis and Kollias, 2001). We compared cytokine expression profiles between AA-affected and normal C3H/HeJ mouse and rat skin, and found that TNF-α/β, IL-12, and IFN-γ were more abundantly expressed in AA-affected rodents (data not shown). It is not clear, however, whether decreased expression of TNF-α/β, IL-12, and IFN-γ is directly related to the restoration of the autoimmune-arrested follicular activity in AA. The cytokine profiles in the two rodent models according to different topical treatments are summarized in **Table I**. Among all the cytokines tested, TNF-α and IFN-γ have been consistently modulated by different therapies. It would be interesting to determine whether any of them play a direct role in therapeutically induced hair regrowth in AA-affected animal models by using specifically targeted antibodies or anti-sense molecules.

Recently emerging transcripteomics and proteomics are a potentially revolutionary breakthrough in determining the molecular mechanisms of disease and therapeutic actions. Our studies have shown that topical anthralin and mechlorethamine are very effective in restoring the activity of autoimmune-induced arrest of follicles in DEBR rats and C3H/HeJ mice, respectively. The accessibility and visibility of skin and hair follicles provide convenience and accuracy in correlating therapeutic efficacy to molecular events. At molecular levels, genes responsible for follicular activity and cutaneous immune function in AA patients should be similar to those in AA-affected DEBR rats and C3H/HeJ mice. Studying the molecular mechanisms regulating follicular activity in animal models would be of great benefit in the rational design of more refined therapeutic modalities for AA patients.

This work was supported by grants from the Canadian Dermatology Foundation (LT, JS, HL) and the Canadian Hair Research Foundation (LT, HL, JS), the National Alopecia Areata Foundation (JS, JPS, HL, LT), and the National Institutes of Health (AR43801, JPS).

REFERENCES

Arrazola JM, Sendagorta E, Harto A, Ledo A: Treatment of alopecia areata with topical nitrogen mustard. *Intern J Dermatol* 24:608–610, 1985

Becker JC, Varki N, Bröcker EB, Reisfield RA: Lymphocyte-mediated alopecia in C57BL/6 mice following successful immunotherapy for melanoma. *J Invest Dermatol* 107:627–632, 1996

Bernardo O, Tang L, Lui H, Shapiro J: Topical nitrogen mustard in the treatment of alopecia areata. *J Am Acad Dermatol* in press

Bodemer C, Peuchmaur M, Fraitaig S, Chatenoud L, Brousse N, DeProst Y: Role of cytotoxic T cells in chronic alopecia areata. *J Invest Dermatol* 114:112–116, 2000

Carroll JM, Crompton T, Seery JP, Watt FM: Transgenic mice expressing INF-γ in the epidermis have eczema, hair hypopigmentation, and hair loss. *J Invest Dermatol* 108:412–422, 1997

Chodorowska G: Plasma concentration of IFN-gamma and TNF-alpha in psoriatic patients before and after local treatment with dithranol ointment. *J Eur Acad Dermatol Venereol* 10:147–151, 1998

Cope AP: Regulation of autoimmunity by proinflammatory cytokines. *Curr Opin Immunol* 10:669–676, 1998

Cork MJ, Crane AM, Duff GW: Genetic control of cytokines: cytokine gene polymorphisms in alopecia areta. *Dermatol Clin* 14:671–178, 1996

Cork MJ, Tarlow JK, Clay FE, *et al*: An allele of the interleukin-1 receptor antagonist as a genetic severity factor in alopecia areata. *J Invest Dermatol* 104:15S–16S, 1995

Duvic M, Lemak NA, Redman JR, Eifel PJ, Tucker SL, Cabanillas FF, Kurzrock R: Combined modality therapy for cutaneous T-cell lymphoma. *J Am Acad Dermatol* 34:1022–1029, 1996

Engert A, Wolf J, Dieh V: Treatment of advanced Hodgkin's lymphoma: standard and experimental approaches. *Sem Hematol* 36:282–289, 1999

Esteve E, Bagot M, Joly P, *et al*: A prospective study of cutaneous intolerance to topical mechlorethamine therapy in patients with cutaneous T-cell lymphomas: French Study Group of Cutaneous Lymphomas. *Arch Dermatol* 135:1349–1353, 1999

Feldmann M, Maini RN: Anti-TNF-α therapy of rheumatoid arthritis: what we have learned? *Ann Rev Immunol* 19:163–196, 2001

Fiedler-Weiss VC, Buys CM: Evaluation of anthralin in the treatment of alopecia areata. *Arch Dermatol* 123:1491–1493, 1987

Firestein GS: Novel therapeutic strategies involving animals, arthritis, and apoptosis. *Curr Opin Rheumatol* 10:236–241, 1998

Freyschmidt-Paul P, Sundberg JP, Happle R, McElwee KJ, Metz S, Boggess D, Hoffmann R: Successful treatment of alopecia areata-like hair loss with the contact sensitizer squaric acid dibutylester (SADBE) in C3H/HeJ mice. *J Invest Dermatol* 113:61–68, 1999

Freyschmidt-Paul P, Ziegler A, McElwee KJ, Happle R, Kissling S, Sundberg JP, Hoffmann R: Treatment of alopecia areata in C3H/HeJ mice with the topical immunosuppressant FK506 (Tacrolimus). *Eur J Dermatol* 11:405–409, 2001

Gilhar A, Landau M, Assy B, Shalaginov R, Serafimovich S, Kalish RS: Mediation of alopecia areata by coorperation between $CD4^+$ and $CD8^+$ T lymphocytes. *Arch Dermatol* 138:916–922, 2002

Gilhar A, Landau M, Assy B, Shalaginov R, Serafimovich S, Kalish RS: Melanocyte-associated T cell epitopes can function as autoantigens for transfer of alopecia areata to human scalp explants on $Prkd^{scid}$ mice. *J Invest Dermatol* 117:1357–1362, 2001

Gilhar A, Shalaginov R, Assy B, Serafimovich S, Kalish RS: Autoimmune hair loss (alopecia areata) transferred by T lymphocytes to human scalp explants on SCID mice. *J Clin Invest* 101:62–67, 1998

Harris DR: Old wine in new bottles. The revival of anthralin. *Cutis* 62:201–203, 1998

Hoffmann R, Happle R: Does interleukin-1 induce hair loss? *Dermatol* 191:273–275, 1995

Hoffmann R, Happle R: Topical immunotherapy in alopecia areata. What, how, and why? *Dermatol Clin* 14:739–744, 1996

Hoffmann R, Wenzel E, Huth A, van der Steen P, Schäufle M, Henninger H, Happle R: Cytokine mRNA levels in alopecia areata before and after treatment with contact allergen diphenylcyclopropenone. *J Invest Dermatol* 103:530–533, 1994

Joosten LAB, Lubberts E, Helsen MNA, van den Berg WB: Dual role of IL-12 in early and late stages of murine collagen type II arthritis. *J Immunol* 159: 4094–4102, 1996

Kassiotis G, Kollias G: TNF and receptors in organ-specific autoimmune disease: multi-layered functioning mirrored in animal models. *J Clin Invest* 107: 1507–1508, 2001

Lange RW, Germolec DR, Foley JF, Luster MI: Antioxidants attenuate anthralin-induced skin inflammation in BALB/c mice. Role of specific proinflammatory cytokines. *J Leuk Biol* 64:170–177, 1998

McDonagh AJG, Messenger AG: The pathogenesis of alopecia areata. *Dermatol Clin* 14:661–670, 1996

McElwee KJ, Boggess D, King LE, Sundberg JP: Experimental induction of alopecia areata-like hair loss in C3H/HeJ mice using full-thickness skin grafts. *J Invest Dermatol* 111:797–803, 1999b

McElwee KJ, Rushton DH, Trachy R, Oliver RE: Topical FK506: a potent immunotherapy for alopecia areata? Studies using the Dundee experimental bald rat model. *Br J Dermatol* 137:491–497, 1997

McElwee KJ, Spiers EM, Oliver RF: *In vivo* depletion of $CD8^+$ T cells restores hair growth in the DEBR model for alopecia areata. *Br J Dermatol* 135:211–217, 1996

McElwee KJ, Spiers EM, Oliver RF: Partial restoration of hair growth in the DEBR model for Alopecia areata after *in vivo* depletion of $CD4^+$ T cells. *Br J Dermatol* 140:432–437, 1999c

McElwee KJ, Tobin DJ, Bystryn JC, King LE, Sundberg JP: Alopecia areata: an autoimmune disease? *Exp Dermatol* 8:371–379, 1999a

Michie HJ, Jahoda CAB, Oliver RF, Johnson BE: The DEBR rat: an animal model of human alopecia areata. *Br J Dermatol* 125:94–100, 1991

Mrowietz U, Jessat H, Schwarz A, Schwarz T: Anthalin (dithranol) *in vitro* inhibits human monocytes to secrete IL-6, IL-8 and TNF-α, but not IL-1. *Br J Dermatol* 136:542–547, 1997

Nelson DA, Spielvogel RL: Anthralin therapy for alopecia areata. *Int J Dermatol* 24:606–607, 1985

Oliver RF, Lowe JG: Oral cyclosporin A restores hair growth in the DEBR rat model for alopecia areata. *Clin Exp Dermatol* 20:127–131, 1995

Perret C, Weisner-Menzel L, Happle R: Immunohistochemical analysis of T-cell subsets in the peribulbar and intrabulbar infiltrates of alopecia areata. *Acta Derm Venereol* 64:26–30, 1984

Philpott MP, Sanders DA, Bowen J, Kealey T: Effects of interleukins, colony-stimulating factor and tumor necrosis factor on human hair follicle growth *in vitro*: a possible role for interleukin-1 and tumor necrosis factor-α in alopecia areata. *Br J Dermatol* 135:942–948, 1996

Ramsay DL, Meller JA, Zackheim HS: Topical treatment of early cutaneous T-cell lymphoma. *Hematol Oncol Clin North Am* 9:1031–1056, 1995

Randall VA: Is alopecia areata an autoimmune disease. *Lancet* 358:1922–23, 2001

Safavi KH, Muller SA, Suman VJ, Moshell AN, Melton LJ: Incidence of alopecia areata in Olmstead County, Minnesota 1975 through 1989. *Mayo Clin Proc The* 70:828–633, 1995

Shapiro J: *Hair Loss. Principles of Diagnosis and Management of Alopecia.* London: Martin Dunitz, 2002

Shapiro J, Price VH: Hair regrowth therapeutic agents. *Dermatol Clin* 16:341–356, 1998

Shapiro J, Sundberg JP, Bissonnette R, *et al*: Alopecia areata-like hair loss in C3H/HeJ mice and DEBR rats can be reversed using topical diphencyprone. *J Invest Dermatol Sym Proc* 4:239, 1999

Sundberg JP, Cordy WR, King LE Jr: Alopecia areata in aging C3H/HeJ mice. *J Invest Dermatol* 102:847–856, 1994

Sundberg JP, King LE Jr: Mouse models for the study of human hair loss. *Dermatol Clin* 14:619–632, 1996

Tang L, Cao L, Sundberg JP, Lui H, Shapiro J: Restoration of hair growth in mice with an alopecia areata-like disease using topical anthralin. *Exp Dermatol* 12:1–6, 2003a

Tang L, Cao L, Bernardo O, *et al*: Topical mechlorethamine restores autoimmune-arrested follicular activity in mice with an alopecia areata-like disease by targeting infiltrated lymphocytes. *J Invest Dermatol* 120:400–406, 2003b

Tesch H, Sieber M, Diehl V: Treatment of advanced stage Hodgkin's disease. *Oncol* 60:101–109, 2001

Todes-Taylor NR, Turner BS, Wood BS, Staratte PT, Morhenn VB: T cell subpopulations in alopecia areata. *J Am Acad Dermatol* 11:216–223, 1984

Wiegrebe W, Muller K: Treatment of psoriasis with anthrones-chemical principles, biochemical aspects, and approaches to the design of novel derivatives. *Skin Pharmatol* 8:1–24, 1995

Zhang JG, Oliver RF: Immunohistological study of the development of the cellular infiltrate in the pelage follicles of the DEBR model for alopecia areata. *Br J Dermatol* 130:405–414, 1994